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Intestinal Structure and Function Related to Toxicology

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The study of toxic effects on small intestinal function is complicated by the integration of the activity of the small intestine with the activities of other regions of the GI tract. Also, the barrier and portal functions of the intestine are not as clearly defined as sometimes assumed. The intestinal surface functions as a barrier to the ingress of large quantities of large water soluble molecules. Lipidic substances enter the body quite readily as do small water-soluble molecules. The small intestinal surface is more a portal than a barrier, with its portal functions divided between nonspecific diffusional entry, which depends on physical properties and electric charge, and entry by specific membrane transport, which depends upon chemical structure. The implications of these properties of the small intestine for toxicological studies are stressed.

In approaching the question of intestinal structure and function related to toxicology, it was very difficult to decide where to begin and where to stop. The barrier and portal functions of the intestines viewed in isolation seemed easy enough to encompass, but the intestines never function in isolation except in the research laboratory. In animals, they are functionally integrated units of the GI tract and, in fact, of the body as a whole. What happens elsewhere influences what happens in the intestines. What happens in the intestines has its consequences in other parts of the body. And it seemed to me important for the toxicologist always to keep this in mind. To further this end, a simple, contracted list of factors in intestinal function is given in Table 1. The list is not intended to be comprehensive, nor does the order of listing reflect importance or complexity. The list should, however, be a reminder that an observed toxicological effect on gross intestinal function may, in fact, not be a direct effect on intestinal structure or function. It may result from an imbalance or altered function elsewhere which in turn may produce profound alterations of intestinal function.

As convenient examples, one might think of the effects of a stimulated release of GI hormones such as gastric inhibitory peptide (GIP) which may reduce jejunal absorption of ions and water (*1*) or of vasoactive inhibitory peptide which may produce active secretion (*2*) or the effects of a reduction in bile salt secretion by the liver. Some of the other papers will serve to sharpen this general perception; thus it is not necessary to dwell in depth on issues at the level of integrated physiology. We may move on to consider the structure and function of the intestines at a cellular and molecular level.

The intestines are a barrier to ingested environ-

Table 1. Factors in intestinal function.

Type of food
Rate of input
Stomach emptying
Adequacy of secretions
Biliary
Pancreatic
Digestive-absorptive capacity
Per unit surface
Per total surface
Residence (or transit) time
Motility
Length
Hormones and drugs
Activators
Inhibitors
Nonabsorbable materials
Neural effects
Cell turnover and differentiation
Rhythms
Bacteria
Blood flow
Disease

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mental substances, some of which may be noxious, and they are a specific, highly active portal for the entry into the body of nutrients and foodstuffs. However, their properties as a barrier or as a portal vary considerably, depending on whether it is the duodenum, the jejunum, the ileum, or the colon that is being considered. In order to stay within reasonable bounds of space, it is only the jejunum and the ileum which will be considered here in detail. To some degree, the principles uncovered may be extrapolated to the specialized situation elsewhere preferably with the aid of a recent review (*3*).

The small intestine where the absorption of nutrients takes place is a tube connecting to the stomach at its upper end and to the large intestine at its lower. In the human adult, the tube is about 280 cm (9 ft) in length and an average of 4 cm (1.5 in.) in internal diameter. The area of the inner surface of the tube is much greater than these measurements suggest because the mucosal surface is heavily folded, and everywhere on these folds are to be found numerous projections called villi. Villi are readily seen under a microscope of low power and there are, in all, 25,000,000. Each villus (Fig. 1) is covered by a sheet of absorptive epithelial cells punctuated at intervals by goblet cells which supply protective mucous. Between the villi are crypts within which the villus cells are produced and from which they migrate outward along the surface of a villus during a short 3-4 days of active life before being extruded into the lumen of the gut, where they disintegrate and are digested. Since the cells differentiate during their stay on the villus, a point for toxicologists is that agents which act to speed up this process may result in an immature and less potent population of cells.

The villus is the working unit of the intestine. It is on the villus that the inner ends of the absorptive cells are brought into close proximity to the blood and lymph which must pick up absorbed nutrients and carry them to other parts of the body. The outer ends of the absorptive cells are in contact with the contents of the intestine and are specialized to perform their work. The outer end of each cell is a "brush border" made up of closely packed, parallel cylindrical processes called microvilli. The limiting plasma membrane of the cell, the brush border membrane, follows the contours of the microvilli. Just beneath the brush border, along the sides of the cells, are to be found specialized junctional structures by means of which the absorptive cells are held together into a more or less continuous sheet. The membrane enclosing the inner portion of the cell is called the basolateral membrane. The brush border membrane is a chemically specific barrier and portal for entry, as will be discussed below. However, it is important to emphasize at this point that the barrier and portal

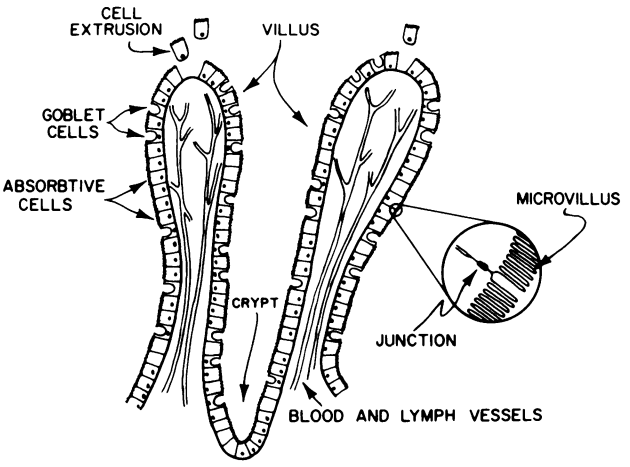

FIGURE 1. Schematic of features of villus architecture and the mucosal lining of the small intestine.

properties of the mucosal lining of the intestine are not solely the barrier and portal properties of the brush border membrane.

First, the space between the microvilli and some of the space extending beyond the villi form a substantial region which does not mix readily with the semifluid contents of the lumen. This so-called unstirred layer is some 400-500 μm in effective thickness (*4*). Molecules diffuse into and through the layer rather than being mixed with it. The effective thickness of the layer can be reduced by increased agitation of intestinal contents. It also seems to be reduced when the villus structure is lost as in active coeliac disease. Assuming the apparent thickness of the layer to be in part due to the coat of mucous on the surface of the intestines, an item to be considered in toxicology would be a possible change in apparent intestinal function brought by a change in the quantity or the physical state of the mucous.

Secondly, the junctions between cells in the small intestine are not tight and thus provide, particularly in the jejunum, a paracellular channel for the movement of ions and small water soluble molecules directly from the lumen to the lamina propria. The effective diameter of the paracellular channels is large enough to permit compounds like salicylate to take this route (*5*). It is also possible that some amounts of nutrients such as glucose and amino acids enter the body by the paracellular channels particularly when their luminal concentrations are high. There are some indications in the literature that this may be the case though comprehensive quantitative studies have not been done (*6*). This possibility is of interest because it could represent a saving of energy in the "downhill" mode of transport while

the concentration of nutrient in the lumen is higher than that in the blood. More particularly for our current concern with toxicology, the availability of the paracellular pathway appears to be influenced by some drugs (*5*).

Thirdly, the extrusion zone at the tip of the villus may be a vulnerable point for entry of substances of considerable size (*7*). The villi of many species contract and relax. During contraction, cells are extruded. During relaxation, water and presumably other materials appropriately placed flow in through this zone.

The brush border membrane (*8*) is a bilayer lipoidal matrix composed of the fatty acid chains of phospholipids and glycosphingolipids interspersed with cholesterol. Inserted in the membrane are upwards of 25 different proteins representing enzyme, transport, and other activities. The enzymes which range upward in molecular weight from 80,000 daltons are held onto the surface of the membrane by hydrophobic tail pieces of about 10,000 daltons. The transport proteins are more generally hydrophobic and appear to span the membrane as would be consistent with their function. The proteins of the membrane appear generally to have carbohydrate chains projecting into the luminal space. There is also a substantial carbohydrate component, especially prominent in the cat, the bat, and man, called the "fuzzy coat," the purpose of which is not known. There are aqueous channels in the membrane through which water, ions and very small water soluble molecules may pass by diffusion. Lipid-soluble molecules of most any size diffuse readily across the matrix of the membrane. Consequently, the brush border membrane is not a barrier for these. However, it is a substantial barrier to the rapid diffusion of large, water-soluble molecules like glucose, because these do not enter the lipoidal matrix and the dimensions of the aqueous channels are too small, being equivalent only to those of pore 3–5 Å in radius.

On the other hand, the barrier and portal properties suggested are not the same throughout life, nor are they adequately explained without considering endocytosis. Macromolecules and even particulates of substantial size are known to be taken up into the epithelial cells (*7*), particularly in the newborn. In the first few days of life, endocytosis is a major process providing in some species a nonspecific route for the uptake of nearly any macromolecule. In others, gamma globulin is taken up rather selectively. The process is less active in the adult animal but still continues. In the adult, particulates appear to be taken up into the lysosomes (*9*), where they may be retained until the cell is shed. However, some of the particulates taken up by the intestine end up in the reticulo-endothelial system, where their presence may be toxicologically important (*7*).

When one considers together all of the nonspecific routes of entry, paracellular channels, the lipid matrix, and endocytosis, one is brought to wonder what may be the special value of the specific routes of entry which seem largely to be concerned with water-soluble food stuffs and nutrients (which is not to ignore the specific ileal uptake of the B_{12}-intrinsic factor complex). One is led to the thought that these routes are of value because of the possibility they provide for the coupling of uptake to metabolic energy, thus to insure the complete or virtually complete capture of foodstuffs which during the evolution of intestinal function were in short supply. This thought could apply as well to the absorption of bile salts, because the energy expended in their synthesis would thus be conserved.

Some support for this general notion would seem to be found when one considers the capacity of the gut to absorb and when one looks for mechanisms which may exert a degree of control which could alter in a substantial way the proportion of food intake absorbed and utilized. So far as I can tell, there is no effective control in the intestine, though there are, of course, known mechanisms, including diurnal rhythms, which can increase or decrease digestion and absorption several fold. To illustrate the point, we may take the absorption of glucose as an example. Some years ago the absorption capacity of a 30 cm segment of intestine in normal humans was measured by Holdsworth and Dawson. From their measured values, it was a simple calculation to arrive at a 24-hr absorptive capacity of 22 lb sugar, representing 50,000 calories (*10*). Such a capacity for sugar absorption is 10 times more than enough to provide for even the most unreasonable individual caloric requirements. Since foods in addition to sugars are also eaten and can contribute independently to the caloric supply, the conclusion drawn above seems inevitable, i.e., control of digestion and absorption is clearly not applied at the level of the intestine. Some control is exerted by a negative feedback mechanism involving receptors in the upper intestine and the motility of the stomach, but this mechanism does not severely limit the ability of an individual to take in food. It has been found, for example, that in jejuno-ileal bypass operations for refractory obesity, approximately 90% of the total length must be bypassed in order to achieve a satisfactory degree of weight loss, and even this limited success may be due more to a reduction of appetite than to a loss of digestive absorptive capacity.

To turn attention now to the specific routes, how they are energized and what is their efficiency, it is first necessary to return to a consideration in slightly

more detail of the enzyme activities attached to the outer surface of the brush border membrane. The peptidases and carbohydrases listed in Table 2 subserve the terminal digestion of products of pancreatic enzyme activity or the digestion of ingested similar foodstuffs and provide directly in the same microenvironment the substrates for a number of the Na^+-dependent transport systems listed in Table 3. Among the major water-soluble foodstuffs, only fructose appears not to benefit from this mode of energy coupling (*8*).

Table 2. Brush border enzymes.

Peptidases
Dipeptidase
Oligopeptidase
γ-Glutamyl transpeptidase
Enterokinase
Carbohydrases
Glucoamylase
Maltase
Lactase
Phlorizin hydrolase (glycosylceramidase)
Sucrase
Isomaltase (α-dextrinase)
Trehalase
Others
Alkaline phosphatase
Guanylate cyclase

Table 3. Na^+-dependent processes for energized absorption.

Amino acids
Ascorbic acid
Bile salts
Biotin
Dipeptides
Folate
Glucose and galactose
Myo-inositol
Phosphate
Riboflavin
Thiamine

In any case, including that of fructose, the organization of the enzymes and the receptor functions of the transporters in the same microenvironment at the surface of the membrane appears to provide, at least for sugars, a kinetic advantage for absorption of the products of the digestive enzymes. These products released at the membranes are better absorbed than the same substance provided free in the lumen.

The Na^+-dependent transporters listed in Table 3 function as indicated in Figure 2. The substrate (glucose, amino acid, or whatever) and Na^+ ion both add

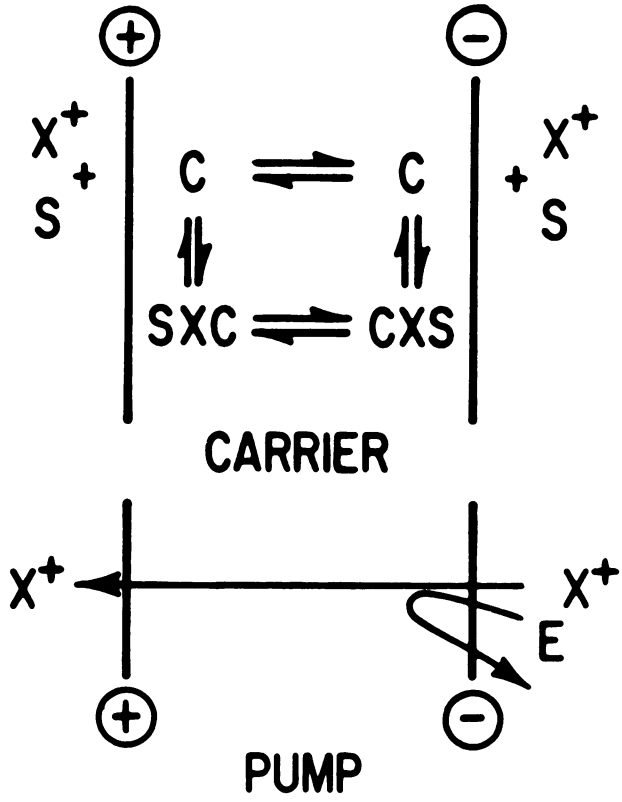

FIGURE 2. Model of ion-dependent active transport. $X^+ = H^+$ or Na^+.

to the "carrier" to form a ternary complex, CNA^+S, which carries, we believe, a positive charge (*11*). This ternary complex may then respond to the forces in the gradient of Na^+ concentration, $\Delta\mu Na^+$ and the membrane potential $\Delta\Psi$ to provide for the accumulation of the substrate within the cell to the limits of the energy available in the total electrochemical potential gradient

$$\bar{\mu}Na^+ = \Delta\Psi + RT \ln ([Na^+]_o/[Na^+]_i)$$

The membrane potential and the chemical gradient of Na^+ both are provided by the operation of an Na^+ K^+ ATP-dependent pump in the basolateral membrane as indicated in Figure 3. The compounds accumulated within the cell are released across the basolateral membrane through specific portals, at least in the case of sugars.

The same forces apparent in Figure 3 are used for the transmembrane movement of other substances. For example, coupling to the Na^+ gradient appears to explain both Cl^- uptake by the intestine and Cl^- secretion by the colon (*12*). Na^+ and other cations may move across the brush border membrane into the cell impelled by the membrane potential (*12*).

Thus, with these limited examples, it is clear that

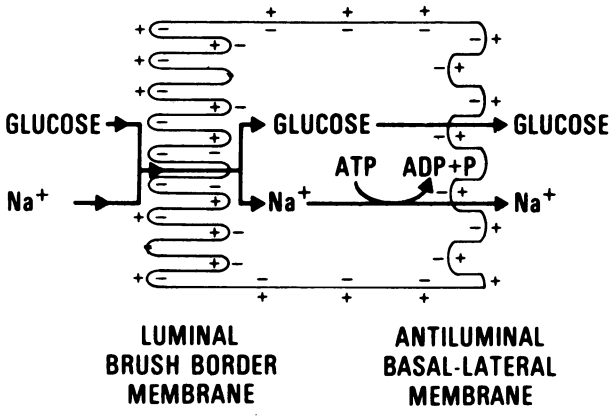

FIGURE 3. Origin and distribution of the Na^+ gradient and the membrane potential in the intestinal cell.

toxicological effects of the epithelial cells to reduce the activity of the basolateral Na^+ pump and/or the membrane potential may have profound effects on absorption and secretion by the intestine. In addition, regional effects on the villus need to be considered. There is plenty of evidence to suggest (*8, 13*) that ion secretory activities from the crypt region of the villus together with the ion absorptive activities toward the tip provide an external fluid circuit which may power absorptive activities especially those, such as fructose, which are not directly energy-coupled at the brush border membrane. Interruption or disproportionation of the external fluid circuit by toxicological actions at the crypts may lead to major disturbance of overall intestinal function.

From what has been presented, the more obvious toxicological targets at the molecular level may be identified as the enzymes, the transporters, and the pumps. However, there is some indication that toxicological action may on occasion be more subtle. For example, some plant lectins are cytotoxic, presumably because they bind to the carbohydrate moiety of membrane proteins. In at least one case (*14*), a severe inhibition of membrane transport processes has been identified. For another example, anionic and cationic surfactants at low concentrations may insert into the membrane and alter the charge density in the vicinity of an enzyme, an ion channel or an ion dependent transporter (*15*). At higher concentrations surfactants may selectively remove some of the functional proteins, they may increase permeability and finally, of course, they may disrupt entirely the membrane structure.

The practical problem created by the complexity of organization which is available for disruption by toxicological agents, is how specifically to isolate and to study the possible events in manageable form. Others here will provide recipes for other aspects of intestinal function. We can provide a tool for the study of the brush border membrane. Following Hopfer's initial success (*16*), our laboratory developed a simple means for the preparation of reasonably pure brush border membrane vesicles from a variety of mammalian species (*17*). We have recently simplified this method (*18*) and have found that it is applicable to a marine species as remote from man as the shark (*19*). From these observations it would seem to be credible to develop a screening program for environmental toxicological agents which may act on one or another aspect of brush border membrane function.

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REFERENCES

1. Helman, C. A., and Barbezat, G. O. The effect of gastric inhibitory peptide on human jejunal water and electrolyte transport. Gastroenterology 72: 376 (1977).
2. Bloom, S. R. Gastrointestinal hormones. In: Gastrointestinal Physiology, II, R. K. Crane, Ed., University Park Press, Baltimore, 1977.
3. Phillips, S. F., and Devroede, G. J. Functions of the large intestine. In: Gastrointestinal Physiology, III, R. K. Crane, Ed., University Park Press, Baltimore, 1979.
4. Winne, D. Dependence of intestinal absorption *in vivo* on the unstirred layer. Naunyn-Schmiedeberg's Arch. Pharmacol. 304: 175 (1978).
5. Barnett, G., Hiu, S., and Benet, L. Z. Effects of theophilline on salicylate transport in isolated rat jejunum. Biochim. Biophys. Acta 507: 517 (1978).
6. Rinaldo, J. E., Jennings, B. L., Frizzell, R. A. and Schultz, S. G. Effects of unliteral sodium replacement on sugar transport across *in vitro* rabbit ileum. Am. J. Physiol. 228: 854 (1975).
7. LeFevre, M. E., and Joel, D. D. Intestinal absorption of particulate matter. Life Sci. 21: 1403 (1977).
8. Crane, R. K. Digestion and absorption: water-soluble organics. In: Gastrointestinal Physiology, II, R. K. Crane, Ed., University Park Press, Baltimore, 1977.
9. Porter, K. R., Independence of fat absorption and pinocytosis. Fed. Proc. 28: 35 (1969).
10. Crane, R. K. Intestinal absorption of sugars. In: Physiological Effects of Food Carbohydrates, A. Jeanes and J. Hodge, Eds., American Chemical Society, Washington, 1975.
11. Crane, R. K., and Dorando, F. In: Function and Molecular Aspects of Biomembrane Transport, E. Quagliarello, et al., Eds., Elsevier/North Holland, 1979, p. 271.
12. Frizzell, R. A., and Schultz, S. G. Models of electrolyte absorption and secretion by gastrointestinal epithelia. In: Gastrointestinal Physiology, III, R. K. Crane, Ed., University Park Press, Baltimore, 1979.
13. Hendrix, T. R., and Paulk, H. T. Intestinal secretion. In: Gastrointestinal Physiology, II, R. K. Crane, Ed., University Park Press, Baltimore, 1977.
14. Li, E., and Kornfeld, S. Effects of wheat germ agglutinin on membrane transport. Biochim. Biophys. Acta 469: 202 (1977).
15. Wojtcsak, L., and Nalecz, M. Surface charge of biological membranes as a possible regulator of membrane-bound enzymes. Eur. J. Biochem. 94: 99 (1979).

16. Hopfer, U., Nelson, K., Perrotto, J., and Isselbacher, K. J. Glucose transport in isolated brush border membranes from rat small intestine. J. Biol. Chem. 248: 25 (1973).
17. Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B. K., Cerda, J. J. and Crane, R. K. Purification of the human intestinal brush border membrane. Biochim. Biophys. Acta 323: 98 (1973).
18. Malathi, P., Preiser, H., Fairclough, P., Mallett, P. and Crane, R. K. A rapid method for the isolation of kidney brush border membranes. Biochim. Biophys. Acta 554; 259 (1979).
19. Crane, R. K., Boge, G., and Rigal, A. Isolation of brush border membranes in vesicular form from the intestinal spiral valve of the small dogfish (*Scyliorhinus canicula*). Biochim. Biophys. Acta 554: 264 (1979).